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*J Immunol* 2010; 184:3648-3655; Prepublished online 5 March 2010; doi: 10.4049/jimmunol.0903346
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GP41-Specific Antibody Blocks Cell-Free HIV Type 1 Transcytosis through Human Rectal Mucosa and Model Colonic Epithelium

Ruizhong Shen,* Ernesto R. Drelichman,† Diane Bimczok,* Christina Ochenbauer,‡ John C. Kappes,‡ Jamie A. Cannon,† Daniela Tudor,§ Morgane Bomsel,§ Lesley E. Smythies,* and Phillip D. Smith*§

Monostratified epithelial cells translocate HIV type 1 (HIV-1) from the apical to the basolateral surface via vesicular transcytosis. Because acutely transmitted HIV-1 is almost exclusively CCR5-tropic and human intestinal epithelial cells preferentially transcytose CCR5-tropic virus, we established epithelial monolayers using polarized HT-29 cells transduced to express CCR5, and an explant system using normal human rectal mucosa, to characterize biological parameters of epithelial cell transcytosis of HIV-1 and assess antiviral Ab blockade of transcytosis. The amount of cell-free HIV-1 transcytosed through the epithelial monolayer increased linearly in relation to the amount of virus applied to the apical surface, indicating transcytosis efficiency was constant (r² = 0.9846; p < 0.0001). The efficiency of HIV-1 transcytosis ranged between 0.05 and 1.21%, depending on the virus strain, producer cell type and gp120 V1–V3 loop signature. Inoculation of HIV-1 neutralizing Abs to the immunodominant region (7B2) or the conserved membrane proximal external region (2F5) of gp41 or to cardiolipin (IS4) onto the apical surface of epithelial monolayers prior to inoculation of virus significantly reduced HIV-1 transcytosis. 2F5 was the most potent of these IgG1 Abs. Dimeric IgA and monomeric IgA, but not polymeric IgM, 2F5 Abs also blocked HIV-1 transcytosis across the epithelium and, importantly, across explanted normal human rectal mucosa, with monomeric IgA substantially more potent than dimeric IgA in effecting transcytosis blockade. These findings underscore the potential role of transcytosis blockade in the prevention of HIV-1 transmission across columnar epithelium such as that of the rectum.

HIV type 1 (HIV-1) enters the host through the mucosa in all transmissions, except those acquired parenterally (1–3). During this process, a key entry event is the translocation of virus across the epithelium. In intestinal, colonic, rectal, and endocervical mucosa, the epithelium is composed of a single layer of polarized, columnar epithelial cells with tight junctions that separate the cells into apical and basolateral domains (4). In contrast, ectocervical and vaginal epithelium is composed of pluristratified epithelial cells that lack a polarized plasma membrane and tight junctions, allowing a rich network of intraepithelial dendritic cells and Langerhans cells to integrate into the epithelium (3–5). Depending on the site of inoculation, these distinct structural features of mucosal epithelium permit the translocation of HIV-1 by different pathways, including columnar epithelial cell transcytosis (4, 6, 7) and dendritic/Langerhans cell transport (5, 8, 9).

Transcytosis is a rapid, nondegradative process in which “cargo” is transported in vesicles from the apical to basolateral surface of polarized cells (6, 10, 11). HIV-1 transcytosis across gut and genital epithelium has been reported to involve viral components, including gp41 (7, 12–14), gp120 (10), and gp160 (10, 11), and host epithelial cell receptor and attachment molecules, including the glycosphingolipid galactosylceramide (12), the coreceptor CCR5 (15), and the heparin sulfate proteoglycan attachment receptors, syndecan (10) and agrin (16). Elucidating the biology of HIV-1 transcytosis is important because disruption of the transcytotic process may provide a strategy for inhibiting HIV-1 entry through columnar epithelium and protection against infection. In this regard, the transcytosis of cell-associated HIV-1 can be inhibited in vitro by dimeric IgA (dIgA) and pentameric IgM (pIgM) isolated from HIV-1-infected subjects (7), secretory IgA specific for gp41 (13, 17), dIgA and pIgM 2F5 and 2G12 Abs (18), mucosal and serum IgA from HIV-1-exposed seronegative individuals (14, 19), and IgG and secretory IgA anti-gp160 (11, 20).

The transcytosis of cell-free HIV-1 across the epithelium has received less investigative attention, although cell-free virus in infected breast milk (21–23) and semen (24–26) likely enters the mucosa of the inoculated recipient through epithelial cell transcytosis. Abs to host cell epitopes such as CCR5 and GalCer block cell-free HIV-1 transcytosis across model epithelium and primary epithelial cells (10, 11, 27), but neutralizing Abs to HIV-1 components reportedly do not block the transcytosis of cell-free virus across HEC-1 cells, which are derived from genital endometrial adenocarcinoma (28). In this study, we characterized the transcytosis of
cell-free HIV-1 through HT-29 cells derived from colon adenocarcinoma epithelium, Ab blockade of HIV-1 transcytosis through the cells, and 2F5 Ab isotype blockade of HIV-1 transcytosis into explanted rectal mucosa.

**Materials and Methods**

**HIV-1 molecular clones and viruses**

Replication competent clones of R5 viruses, including YU2 (29), NA20.B59, NA353.B27, NA420.B33, and NA420.LN85 (30–32), were prepared by transfection of plasmid DNAs into 293T cells using Fugene 6 (Roche, Indianapolis, IN) per the manufacturer’s protocol. After 60 h, the supernatants were harvested, clarified by low-speed centrifugation, filtered through a 0.45 μm filter, aliquoted and stored at −80 °C. Viruses were titrated using TZM-bl cells (27), and p24 levels were measured by ELISA (PerkinElmer, Boston, MA). The X4 viruses SG3 and NL4-3 were prepared similarly using pSG3 (33) and pNL4-3 plasmids (34), respectively. The YU2 and SG3 plasmids were obtained from Dr. George Shaw, University of Alabama at Birmingham; NA20.B59, NA353.B27, NA420.B33, and NA420.LN85 molecular clones from Dr. Paul Clapham, University of Massachusetts; and pNL4-3 from the AIDS Research and Reference Reagent Program, National Institutes of Health. The replication competent NL4-3.Balecto was constructed by replacing the ectodomain of the Env gene of pNL4-3 with that of the R5 virus BaI and produced as previously described, resulting in a virus with a X4 backbone and an R5 ectodomain (T. Edmonds, C. Ochsenbauer, and J.C. Kappes, submitted for publication). NL4-3.Balecto was used to assess Ab inhibition of HIV-1 transcytosis as part of a larger (Center for HIV/AIDS Vaccine Immunology [CHAVI], National Institutes of Health) multilaboratory investigation of the inhibition of HIV-1 entry in different systems. To examine the effects of viral producer cells on HIV-1 transcytosis, NL4-3.Balecto was propagated in MT-4/R5 cells and PBMCs, and BaI was grown in PBMCs. SF162 and SHIVSF162P3 were provided by CHAVI.

**Abs**

Abs used to evaluate blockade of HIV-1 entry included 2F5 IgG1 (35), 2F5 IgG2a, 2F5 IgG2b (18), 2F5 IgG3 (32), 13H11 (36, 39), 5A9 (39), PA-3F11 mouse antibody to the gp120 ELDKWA peptide, as expected, and bound to gp41-membrane proximal region of HIV-1 in RPMI supplemented with 10% FCS. The Ab was specific for the Env ectodomain (T. Edmonds, C. Ochsenbauer, and J.C. Kappes, submitted for publication). The Ab was specific for the Env ectodomain and was determined to be a nonneutralizing Ab.

Table I. **Abs used to assess inhibition of HIV-1 transcytosis**

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<tr>
<th>Ab Brief Description</th>
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<tr>
<td>2F5 IgG1</td>
<td>Human cluster II mAb to gp41 MPER, binds ELDKWA</td>
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<tr>
<td>2F5 IgG2a</td>
<td>Human cluster II mAb to gp41 MPER, binds ELDKWA</td>
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<td>2F5 IgG3</td>
<td>Human cluster II mAb to gp41 MPER, binds ELDKWA</td>
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<td>5A9</td>
<td>Mouse cluster II anti-gp41 MPER region mAb, HIV-1 non-neutralizing</td>
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<tr>
<td>PA-3F11</td>
<td>Mouse antianthrax protective Ag mAb</td>
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<td>Control IgM</td>
<td>Polyclonal Ab from pooled normal human serum</td>
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**Model columnar epithelium**

Epithelial monolayers were established in transwell chambers using HT-29 cells (American Type Culture Collection, Manassas, VA) transduced to constitutively express CCR5. The level of CCR5 expression (15% CCR5⁺, relative mean fluorescence intensity 485) in the HT-29 cells used here was determined by flow cytometry using anti-CCR5 mAb 2D7 (BD Bioscience, San Jose, CA). To establish the HT-29 monolayers, 3 × 10⁵ HT-29 CCR5⁺ cells in 100 μl media were transferred to a 6.5-mm diameter transwell in a 24-well plate (Corning, Corning, NY) and allowed to grow into a tight, polarized monolayer on the transwell polycarbonate membrane containing 3.0 μm pores. The monolayer formed apical and basolateral surfaces that mimicked the luminal and basolateral domains of columnar epithelium in vivo. Monolayer integrity was measured using transepithelial electrical resistance with a volt-ohm meter (Millipore Millicell-ERS, Millipore, Concord, MA), and monolayers were used only when the transepithelial electrical resistance was 390 mΩ/cm² or greater, consistent with nonpermeable, intact tight junctions.

**Transcytosis assay and transcytosis blockade in model epithelium**

To determine the relationship between input and output virus, HIV-1 (1 × 10⁵ infectious units) was inoculated onto the apical surface of HT-29 monolayers, 2 h later media in the lower chamber was harvested, and the virus that had entered the lower chamber was titrated using TZM-bl cells. Transcytosis efficiency was calculated by dividing the amount of virus (in infectious units) in the lower chamber by the amount of virus inoculated onto the apical surface in the upper chamber.

To analyze Ab blockade of transcytosis, Abs 2F5 5-fold serial dilutions up to 50 μg/ml were first applied to the apical surface and incubated for 30 min after which virus (11 ng p24, which equals to 3 × 10⁵ infectious units) was added to the apical chamber and incubated for 2 h, mimicking the in vivo inoculation of virus onto a mucosal surface containing previously secreted Ab. Virus in the lower chamber was harvested and quantified by p24 ELISA to measure inhibition of virus transcytosis and not neutralization. In the indicated experiment, Abs were added to the lower chamber and incubated for 1 h before adding virus to the upper chamber. Ab inhibition of HIV-1 transcytosis was expressed as relative transcytosis efficiency, with transcytosis efficiency in the presence of isotype-matched control Ab defined as 100%.

**Rectal mucosal explant**

The proximal (internal) portion of normal rectum obtained from donors undergoing elective perineal proctectomy for rectal prolapse was used to construct mucosal explants, as we recently described for small intestinal and vaginal explants (45, 46). Histological analysis of adjacent tissue confirmed that the rectal epithelium was intact and the lamina propria not inflamed. Briefly, the submucosa was removed by mechanical dissection, the resultant mucosa was sectioned into 1 cm² pieces, and each piece was immediately attached to a disk of filter paper with a central 0.5-cm circular perforation, with the intact apical surface in the superior position to the filter paper. The filter paper then was sealed at the outer edge to the nylon mesh of a 40 μm cell strainer (BD Falcon, Bedford, MA) with surgical glue. A polystyrene cylinder (1 cm high, 0.6 cm diameter), hereafter referred to as the “upper well,” was attached with surgical glue to the apical surface of the mucosa, and the mucosal surface in the upper well was bathed in 50 μl RPMI to maintain epithelial cell viability. The explant...
then was placed in a TC-6 well plate and cultured at 37°C in RPMI containing 10% human AB serum (Atlanta Biological, Norcross, GA).

To measure 2F5 Ab inhibition of HIV-1 transcytosis across rectal epithelium in the mucosal explant, Abs were added to the upper well and incubated for 30 min prior to the addition of YU2 (55 ng p24) to the well. After a 2 h incubation at 37°C, explants were harvested, washed three times with PBS, trypsinized for 10 min, and washed three more times with PBS. DNA-free total RNA was isolated using the Qiagen Plus Mini RNAeasy kit per the manufacturer’s manual and analyzed by quantitative RT-PCR to measure apically applied virus that had entered and remained in the mucosa. Abs inhibition of HIV-1 entry into the rectal mucosa was expressed as relative transcytosis efficiency as described previously.

**Real-time RT-PCR**

HIV-1 RNA was assessed by real-time PCR, using our previously described protocol (45). Briefly, total RNA was transcribed into cDNA using the iScript cDNA Synthesis Kit (BioRad, Hercules, CA) with oligo (dT) and random primers, and the gag gene was amplified with TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA) (47). The gag-specific primers included 5’-ACATCAAGCGCCATGCAAAT-3’ and 5’-CTATGTCACCTCCCCTTTGTTCCT-3’. The gag-specific probe was 5’-6-FAM-ACCATCAATGGAAGCTGCAATGGG-TAMRA-3’. Expression of the endogenous housekeeping gene 18S rRNA (Ref. Seq. X01520.1) was determined simultaneously with VIC/TAMRA labeled primer-probe set. Real-time PCR reactions were run for 50 cycles (15 s 95°C, 60 s 57.9°C) on a Chromo4 PCR system (BioRad) and analyzed with Opticon Monitor software, version 3.1. Relative copy numbers were determined based on a standard curve and normalized to 18S rRNA.

**Statistics**

Values of \( p \) were calculated using Student \( t \) test.

**Results**

**Parameters of HIV-1 transcytosis through columnar epithelium**

Because transmitted and founder viruses are almost exclusively CCR5-tropic (48) and human primary intestinal epithelial cells preferentially translocate CCR5-tropic viruses (27), we established HT-29 CCR5+ epithelial monolayers to investigate the biological properties of R5 virus transcytosis. We first examined whether the amount of HIV-1 that transcytosed through the model epithelium increased linearly in relation to the amount of input virus (Fig. 1A), indicating that the efficiency of transcytosis was constant \((r^2 = 0.9846, p < 0.0001)\) by linear regression). To determine whether HIV-1 transcytosis is dependent on the virus strain, equal amounts of X4 viruses SG3 and NL4-3 and R5 viruses NL4-3.Balecto and YU2 were added in parallel to the apical surfaces of individual epithelial cell monolayers, and transcytosis efficiency was determined. The efficiency of HIV-1 transcytosis was low; mean transcytosis efficiency \((\pm SD)\) for SG3 was 0.33 ± 0.14% and for NL4-3 0.38 ± 0.07%, NL4-3.Balecto 0.05 ± 0.01%, and YU2 0.36 ± 0.18% (Fig. 1B). To examine the influence of the variable region of gp120 on HIV-1 transcytosis, we assessed the transcytosis efficiency of four macrophage-tropic viruses (NA20.B59, NA353.B27, NA420.B33, and NA420.LN85) that have the same NL4-3 genomic backbone but different R5 envelope genes (31, 32). The transcytosis efficiency of these viruses ranged between 0.32 and 1.21% (NA20.B59: 0.32 ± 0.09%; NA353.B27: 0.54 ± 0.11%; NA420.B33: 1.21 ± 0.32%; and NA420.LN85: 0.50 ± 0.12%) (Fig. 1C), indicating variation in transcytosis efficiency among viruses with different V1–V3 loop signatures.

Enveloped viruses released from infected cells may incorporate host cell proteins internally or embed them into the virion envelope (49). HIV-1, in particular, is reported to acquire distinct envelop glycosylation patterns of the producer cell (50), potently modulating the infectivity of progeny virions (51). Therefore, we evaluated whether the production of HIV-1 by different host cells affects the transcytosis efficiency of the virus through epithelial cells. As shown in Fig. 1D, the transcytosis efficiencies of NL4-3.Balecto produced by 293T cells was 0.06 ± 0.01%, MTR5 cells 0.03 ± 0.02%, and PBMCs 0.14 ± 0.02%, indicating enhanced transcytosis efficiency for virus produced in primary mononuclear cells.

**Effects of HIV-1-specific IgG Abs on HIV-1 transcytosis**

We next investigated the ability of IgG mAbs to HIV-1 to inhibit transcytosis of the virus across HT-29 monolayers. NL4-3.Balecto was used in these experiments to standardize the input virus as part of the multilaboratory CHAV1 investigation of HIV-1 entry blockade. At a concentration of 50 μg/ml, 7B2, an Ab to the immunodominant region of gp41 (39), inhibited the transcytosis of NL4-3.Balecto by 57%, in contrast to 13H11 and 5A9, non-neutralizing Abs to gp41 cluster II MPER (39), which exhibited very limited (<20%) inhibition (Fig. 2). The MPER is a region of gp41 crucial for HIV-1 infection and controls cell-associated HIV-1

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**FIGURE 1.** Transcytosis of cell-free HIV-1 through model epithelium. A. Increasing amounts of NL4-3.Balecto were inoculated onto tight HT-29 monolayers (Input virus), and, 2 h later, media in the basolateral chamber was harvested and analyzed for HIV-1 by titration on TZM-bl cells (Output virus). Transcytosis efficiency across model epithelium was determined for (B) different HIV-1 strains, (C) NL4-3 and molecular clones with the same NL4-3 backbone but different envelope genes, and (D) HIV-1 produced in different cell types. For B–D, the amount of virus added was 1 × 10^5 infectious units. Data are the mean of 3–5 experiments; error bars represent SD. Differences between the bars and other bars was (B) \( p < 0.02 \); (C) \( p < 0.02 \); and (D) \( p < 0.004 \).
transcytosis. Concerning cell-free HIV-1 transcytosis, 2F5 IgG1, which recognizes the conserved epitope ELDKWA (35, 52, 53), was the most potent inhibitor of transcytosis among the mAbs tested. In contrast, 4E10, the broadest HIV-1 neutralizing Ab, which binds the conserved epitope NWFDIT in the gp41 MPER (54, 55), displayed no inhibitory effect, suggesting that the more surface-exposed ELDKWA (56) and not NWFDIT is involved in transcytosis. Interestingly, the IS4 and ISQ Abs to cardiolipin, which neutralize HIV-1 in PBMCs, displayed modest to very low, albeit reproducible, inhibition of NL4-3.Balecto transcytosis, suggesting the cardiolipin epitope is minimally involved in cell free HIV-1 transcytosis. Control Abs to anthrax protective Ag (PA-3F11) and a respiratory syntia virus Ag (Synagis) had no inhibitory effect. Together, these data suggest that separate epitopes are involved in HIV-1 neutralization and transcytosis, agreeing with the findings of Chomont et al. (28) and Matoba et al. (57).

IgG and dIgA, but not plgM, 2F5 Abs substantially reduce HIV-1 transcytosis

Because Ab isotypes to the same epitope may vary in their fine specificity (58) and avidity (59) to certain Ags, we next determined whether IgG1, dIgA, and plgM isotypes of 2F5 also inhibited cell-free HIV-1 transcytosis. The 2F5 Abs at 5-fold serial dilutions were applied to the epithelial surface, followed 30 min later by virus, and 2 h later supernatant in the lower chamber was harvested and virus quantified by p24 ELISA. IgG1 and dIgA, but not plgM, 2F5 Abs inhibited HIV-1 transcytosis through the epithelium in a dose-dependent manner (Fig. 3); 2F5 IgG reduced transcytosis by 66% and dIgA by 61% at a concentration of 50 μg/ml. Even at a low concentration of 0.4 μg/ml, IgG1 and dIgA 2F5 Abs inhibited transcytosis by 45% and 34%, respectively (Fig. 3). The 2F5 Abs added to the basolateral chamber did not inhibit HIV-1 transcytosis (data not shown).

To determine whether 2F5 Ab inhibition of HIV-1 transcytosis depends on the HIV-1 strain, we next measured the efficiency with which a panel of viruses transcytose across HT-29 monolayers in the presence of 2F5 Abs at 50 μg/ml. Both IgG1 and dIgA 2F5 Abs potently inhibited SF162 and NL4-3-Balecto, R5 viruses; IgG1 2F5 Abs reduced transcytosis 22–64% (Fig. 4A) and dIgA 2F5 Abs 16–53% (Fig. 4B). Again, plgM 2F5 had no inhibitory effect on epithelial cell transcytosis of these viruses (data not shown).

**FIGURE 2.** Inhibition of HIV-1 transcytosis across model epithelium by a panel of IgG Abs. Abs at a concentration of 50 μg/ml were applied to HT-29 epithelial monolayers for 30 min prior to the inoculation and incubation (2 h) of HIV-1 NL4-3.Balecto (11 ng p24). Virus transcytosed into the basolateral chamber was quantified by p24 ELISA with transcytosis efficiency in the presence of control Ab defined as 100%. Results are the mean of three experiments; error bars represent SD. Difference between the *bars and control Ab was p < 0.01.

**FIGURE 3.** 2F5 isotype Ab inhibition of HIV-1 transcytosis through model epithelium. A, IgG1, dIgA, and plgM 2F5 Abs at the indicated concentrations were applied to the apical surface of HT-29 epithelial monolayers for 30 min prior to the inoculation and incubation (2 h) of HIV-1 NL4-3.Balecto (11 ng p24). Virus transcytosed into the basolateral chamber was quantified by p24 ELISA with transcytosis efficiency in the presence of control Ab defined as 100%. Results are the mean of three experiments; error bars represent SD. Difference between all bars and the corresponding IgG1 and dIgA control Ab was p < 0.03.

**FIGURE 4.** 2F5 Ab inhibition of transcytosis of multiple isolates of HIV-1 across model epithelium. IgG1 (A) and dIgA (B) 2F5 Ab inhibition of HIV-1 transcytosis was assessed by comparing p24 input and output levels with transcytosis efficiency in the presence of control Ab defined as 100%. The amount of virus added was 3 × 10^5 infectious units. Results are the mean of 3–5 experiments; error bars represent SD. Difference between the *bars and corresponding control Ab was p < 0.05.
mlgA blocked transcytosis by 90% but dlgA by only 41%, and at 0.08 µg/ml, mlgA Abs inhibited transcytosis by 30% but dlgA Abs had no inhibitory effect (Fig. 5). Thus, compared with d lgA 2F5 anti–HIV-1 Abs, ml gA 2F5 Abs more potently reduced HIV-1 transcytosis across model epithelium.

2F5 Abs inhibit HIV-1 entry into rectal mucosa

To more closely mimic mucosal HIV-1 transmission in vivo, we established a leakproof rectal explant system (Fig. 6A) similar to our previously described vaginal and intestinal explant system in which virus transcytoses across columnar epithelium to enter the mucosa (45, 46). Using this system, we determined whether 2F5 Abs blocked HIV-1 transcytosis across rectal epithelium, thus entry into rectal mucosa. Total RNA was isolated from the explanted tissue at the conclusion of the entry assay, subjected to quantitative RT-PCR, and the relative copy number of HIV-1 RNA was determined according to the standard curve method by normalizing to 18S rRNA. Com pared with control Abs, IgG1 and d lgA 2F5 isotype Abs significantly decreased the HIV-1 RNA copy number detected in explanted rectal mucosa (Fig. 6B). The decreased copy number in rectal mucosa from three separate tissues corresponded to IgG1- and d lgA-mediated reductions in the efficiency of HIV-1 transcytosis into rectal mucosa by 47% and 85%, respectively (Fig. 6C). Interestingly, p lgM, which did not inhibit transcytosis in model epithelium (Fig. 2), caused a 20% reduction in transcytosis in rectal mucosa (Fig. 6C). Having shown that ml gA 2F5 Abs more potently inhibited HIV-1 transcytosis across model epithelium ( Fig. 4), we next compared the abilities of ml gA and d lgA 2F5 to block HIV-1 transcytosis across rectal mucosal epithelium (Fig. 7). Compared with control IgA and d lgA, ml gA 2F5 Abs at a final concentration of 2.5 µg/ml significantly decreased the number of HIV-1 RNA copies detected in rectal mucosa (Fig. 7A). ml gA 2F5 inhibited HIV-1 transcytosis and entry into rectal mucosa by 77%, whereas d lgA 2F5 inhibited transcytosis and entry by only 18% (Fig. 7B). Thus, 2F5 isotype Abs, especially ml gA, inhibited HIV-1 transcytosis across rectal epithelium and thus entry into the subepithelial lamina propria.

Discussion

Excluding parenteral transmissions, virtually all HIV-1 infections are acquired via the mucosal surfaces of the genital and gastrointestinal tracts (1–3), underscoring the importance of targeting mucosal transmission pathways, including transcytosis, in the prevention of HIV-1 infection. To model the transcytosis of HIV-1 across columnar epithelial cells (4, 6, 7), we constructed a monolayered epithelium using HT-29 cells transduced to express CCR5 because CCR5-tropic viruses are the dominant viruses transmitted in vivo (48) and are preferentially transcytosed across intestinal epithelial cells in vitro (27). Among the engineered HT-29 cells, 15% expressed CCR5, an expression level comparable to the 12% of normal human intestinal epithelial cells that express CCR5, which we previously reported (27). We identified the following features of transcytosis in this model system. First, the amount of HIV-1 transcytosed across the monolayer increased linearly in relation to the amount of virus inoculated onto the apical surface. Second, the efficiency with which cell-free HIV-1 transcytosed through the epithelial monolayer ranged between 0.05 and 1.21%, depending on the HIV-1 strain. Third, the efficiency of HIV-1 transcytosis varied 4-fold among macrophage-tropic viruses with different envelope genes. Fourth, the cell source of HIV-1 affected transcytosis efficiency, as virus produced by PBMCs transcytosed more efficiently than virus produced by cell lines.

The HIV-1 inoculated onto the epithelial monolayers and explanted rectal mucosa in the experiments reported in this study was cell-free virus. Cell-free HIV-1, along with cell-associated virus, is present in breast milk (9, 50) and semen (1, 24, 26) and...
thus available for transcytosis across recipient small intestinal and rectal columnar epithelium during vertical and homosexual/heterosexual transmission, respectively. Recently, spermatozoa were shown to bind HIV-1 via viral gp120 attachment to the heparan sulfate expressed on the surface of sperm (61), indicating another source of cell-associated virus in semen. Presumably, transcytosis through HT-29 model epithelium is initiated by viral envelope binding to the epithelial surface. In this connection, the V3 region is reported to be important in HIV-1 transcytosis across pluri-stratified vaginal and ectocervical epithelium (10). In this study, we showed that transcytosis efficiency varied among four viruses (NA20.B59, NA353.B27, NA420.B33, and NA420.LN85) with different V1–V3 regions. The charge of the V3 regions for the four viruses was the same, but sequence alignment of the envelopes of these viruses (30) revealed substitution of leucine for the more conserved phenylalanine at residue 315 in the V3 region of NA420.B33, the most efficiently transcytosed of the four viruses, raising the possibility that transcytosis efficiency was dependent, in part, on the V3 aa sequence. However, our findings do not exclude a role for other envelope determinants in HIV-1 transcytosis through columnar epithelium.

Cell-free HIV-1 transcytosis also has been investigated using endothelial cells as a model for heterosexual transmission (11, 28). Using HEC-1 endothelial monolayers with a transepithelial resistance of >300 Ohms/cm², Chomont et al. showed that 13 HIV-1-specific mAbs, including broadly neutralizing 2F5 and gp120-specific 2G12 and IgG1b12, did not inhibit HIV-1 transcytosis (28). Two explanations address the difference between these findings and our results. First, in our system, IgG1 2F5 was preincubated on the epithelial cell surface prior to inoculation of virus to more closely model our results. First, in our system, IgG1 2F5 was preincubated on the epithelial cell surface prior to inoculation of virus to more closely model our results. Second, our HT-29 epithelial cell line, as well as transepithelial resistance and experimental design, are important considerations in assessing Ab inhibition of HIV-1 transcytosis through model epithelium.

Using HT-29 epithelial monolayers, we showed that IgG1 and dIgA, but not plgM, 2F5 Abs applied to the apical surface substantially reduced HIV-1 transcytosis through monolayered epithelium. The addition of Abs to the basolateral chamber had no inhibitory effect, despite the previously demonstrated ability of dIgA and plgM applied to the basolateral surface to neutralize cell-associated HIV-1 intracellularly (7, 18). Importantly, we used p24 to quantify input and output virus in the HT-29 assays, indicating that reduction in measurable virus in the basolateral chamber was due to the inhibition of transcytosis and not virus neutralization.

Finally, we developed an explant system using normal human rectal mucosa to test Ab inhibition of HIV-1 transcytosis across rectal mucosa ex vivo. IgG1 and dIgA isotypes of 2F5 Ab to ELDKWA in the gp41 MPER significantly inhibited HIV-1 transcytosis across the rectal mucosa. Compared with the IgG isotype, dIgA 2F5 displayed more potent inhibition of HIV-1 entry into rectal mucosa, reflecting the role of IgA as the predominant Ig isotype in gut secretions, in contrast to IgG, the dominant Ig isotype in female genital secretions (62, 63). These findings provide support for vaccine strategies directed at eliciting mucosal Abs to the MPER of HIV-1 gp41 (17, 57).

IgA and IgG anti-HIV-1 Abs have been detected in nearly all external secretions (62–64). IgA is actively transported across the epithelium via the polymeric IgR (65) to protect against the entry of certain pathogens. Systemic and intravaginal administration of neutralizing IgG Abs effectively prevented mucosal SHIV infection in macaques (66–68), consistent with the significant contribution of circulating IgG to genital IgG and IgG being the predominant isotype in genital secretions (62, 63). In this study, we report that IgG1 and dIgA 2F5 Abs at 50 µg/ml, and mIgA 2F5 Abs at 2.5 µg/ml, substantially reduced the transcytosis of HIV-1 through human rectal mucosa. (A single concentration of Ab was used due to the limited availability of normal rectal mucosa.) Importantly, mIgA 2F5 Abs inhibited HIV-1 transcytosis across both model epithelium and human rectal mucosa substantially more efficiently than dIgA Abs. The more effective blockade of HIV-1 transcytosis efficiency by mIgA was not due to an increase in avidity but more likely was due to the better access of the smaller IgA molecule to the MPER in the virus spike. The ability of MPER-specific Abs to inhibit HIV-1 transcytosis suggests that this region of the viral envelope plays a crucial role in HIV-1 transcytosis of both cell-free (this study) and cell-associated HIV-1 (13–15). In this regard, the MPER is the key structural component that binds to epithelial cell galactosylceramide, the first step in the transcytosis process (12). Thus, our findings suggest that Ab inhibition of HIV-1 transcytosis is achievable in intact rectal mucosa and should be considered a potential strategy for the prevention of HIV-1 transmission by the rectal route.

Disclosures
The authors have no financial conflicts of interest.

References


